with increasing concentrations of analyte (*e.g.*, a gradient reading). In alternate embodiments, the reaction occurs at one particular or multiple threshold levels, as desired (*See e.g.*, U.S. Patent 5,032,506, incorporated herein by reference in its entirety).

In some embodiments that require a series of chemical reactions to take place in sequence, the assay test further comprises multiple chambers for separating, isolating, combining, or storing the reaction components. For example, when a chemical is stored dry, but active only in aqueous solution, separate chambers store the chemical and aqueous solution. Directly prior to or during use of the assay test, the contents of the chambers are combined (*e.g.*, by breaking a barrier separating the separated components).

In some embodiments of the present invention, the reaction means is immobilized to increase durability, accuracy, and ease of use. For example, in some embodiments the reaction means is immobilized on filter paper, or another material, which allows transfer of the sample to the reaction means and provides a reflective surface for enhanced colorimetric detection. The reaction means may also be immobilized in chambers or in gels. In some embodiments of the present invention, the reaction means is immobilized in a porous metal oxide matrix using the sol-gel method (*See generally*, Brinker and Scherer, Sol-Gel Science, Academic Press, San Diego [1995]). Sol-gel entrapment provides cost-efficient, stable, accurate, reliable, consistent, and robust materials that can be produced in a variety of shapes and sizes. The unique properties of sol-gel materials such as optical transparency, durability, and tailorable properties (*e.g.*, porosity, surface functionalization, thin films, and bulk materials) provide an ideal material for immobilization of colorimetric materials. The sol-gel process has been used for entrapping organic molecules such as dyes and proteins in silica gels (*See e.g.*, Avnir, Accounts Chem. Res. 28: 328 [1995]; Yamanaka *et al.*, Am. Chem. Soc. 117: 9095 [1995]; Miller *et al.*, Non-Cryst. Solids 202: 279 [1996]; and Dave *et al.*, Anal. Chem. 66: 1120A [1994]).

In particularly preferred embodiments, the assay test further comprises an indicator that comprises a second reaction means. The indicator provides a detectable signal indicating the introduction of sufficient sample to the first reaction means for a reaction to take place, ensuring the reliability of the assay test. Several of such preferred embodiments are described in detail below. For example, in one embodiment of the present invention, the indicator is located at the end of a sample path, downstream of the first reaction means. The sample must pass through the first reaction means before reaching the indicator. By providing the indicator with a second reaction means, a positive result with the indicator demonstrates that a sufficient amount of sample has been exposed to the first reaction means. In some embodiments, the indicator employs the same chemistry as the primary assay reaction site, while in other embodiments scavengers are excluded and/or a different chromagen is used (independent of toxicity, irritability, or carcinogenicity if the indicator is not exposed to the user).

In other preferred embodiments, the assay test comprises a protective encasement. In some embodiments, the protective encasement comprises a material such as foil and covers the reaction means. In such embodiments, the protective encasement is automatically broken and reveals the reaction means when the user operates the assay test. In still other embodiments, the protective encasement comprises a material such as foil and surrounds the entire assay test. In such embodiments, the user opens the protective encasement to reveal the assay test before operating the assay test.

B. Operation

In one preferred embodiment of the present invention, a protective storage container is opened to reveal an assay test. The assay test operates by first saturating an absorbent material on one end of the assay test with a sample. Depending on the reaction means used in the assay test, the user waits for a period of time and interprets the detectable signal produced by the reaction means. In embodiments that employ an indicator, to check the reliability of the assay test, the user observes if enough sample

was initially put on the absorbent material by viewing a detectable signal from the indicator. The absence of a detectable signal from the indicator demonstrates that not enough sample was initially put on the absorbent material, and that the test may not be reliable. Finally, the user checks their analyte concentration by viewing a color change or other detectable signal (*e.g.*, the appearance of a symbol such as a shape or word) from the reaction means. In some embodiments, to make the assay test easy to decipher, the user compares the color changes to pictorial and written instructions printed on the assay test or a delivery system.

In another preferred embodiment of the present invention, a protective encasement is opened to reveal an assay test. The assay test operates by first saturating an absorbent material on one end of the assay test with a sample. The user then, in one-step, either (1) folds or slides this saturated end into a well on the second region of the assay test, or (2) folds or slides pieces from a second region of the assay test around or onto the saturated end. In preferred embodiments, the folding or sliding motion is designed for quick and easy use. Detection is carried out as described above.

In some preferred embodiments where an enzyme is used as part of the reaction means, the portion of the test assay containing the reaction means (e.g., a pad on a test strip) is maintained in the mouth of a subject for an extended period of time (e.g., for 5 seconds to several minutes), as the enzymatic reaction proceeds at a higher rate at the elevated temperature in the mouth. This method is in contrast to methods where the test assay is briefly saturated with the saliva and then incubated at room temperature until color development. Where the enzymatic reaction is allowed to occur in the mouth, the entire reaction time from initial testing to detecting the presence or absence of a color change is carried out in less time. The increased speed for reading the assay increases the likelihood that the assays will be used.

One embodiment of the alcohol concentration assay test of the present invention is illustrated in Figure 1. The assay test is approximately 1.5 mm in thickness, and has overall dimensions of roughly 5 cm x 1.25 cm, although both larger and smaller dimensions are contemplated and can be designed, as desired (e.g., a